Assessment of Fungi Associated with African Pear (*Dacryodes Edulis*) Leaves

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Abstract

This study was aimed at surveying fungal organisms associated with the decline of *Dacryodes edulis* leaves found in some trees in Afikpo North L.G.A, Ebonyi State of Nigeria. Foliar symptoms observed included black leaf spots, yellowing white patches and crinkling diseases. The result of the disease sampling in different areas studied showed a high prevalence of diseases in the leaves of *Dacryodes edulis* leaves inspected. Yellowing had highest incidence (29.50%) followed by black leaf spot (24.50%) then crinkling (21.00%) with white patches (9.750%) having the least disease incidence. Disease severity showed that black leaf spot was the most severe of the diseases (29.22%). This was followed by yellowing (21.094%) with white patches (18.437%) and crinkling disease having the least severity on the *Dacryodes edulis* leaves (16.095%). The result when analyzed using analysis of variance showed that the diseases are significant at p = 0.5.

Keywords: *Dacryodes edulis*, leaves, fungi

Introduction

The African pear (*Dacryodes edulis*) grows mostly in the tropics. It grows up to 18m in height and exudes an odoriferous gummy substance from injured or excised portion of the stem (Ekpa, 1993). It is cultivated in most rural communities by peasant farmers for its fruits. The leaves are pinnate with leaflets measuring 3 to 4cm by 2-3cm. The leaflets are glabrous, narrowly oblong and elliptic (Sofowa, 1999). The fruit is red, turning blue-black when ripe with unpleasant turpentine smell. The fruit is oval in shape and matures within the months of May and June. The fruit consist of large seeds, surrounded by thin monocarp. The pulp is boiled, roasted or eaten raw as a dessert fruit. The pulp may also be boiled or roasted to form a kind of butter (Irvine, 2002).

Resins or exudates occur in the genus and resin from some species and is used in African medicine (Leakey, 1999). The stem bark yields a resin or exudate which is also primitive oil. The resin is medicinal and is applied to cure skin diseases such as ringworms, craw-craw, etc. They are also used to treat parasitic organism like tick and jigger (Hutchison, *et al.*, 2003). The exudates are used in food and cosmetics industry as thickeners, flavors, stabilizers (Ekpa, 2000). Diseases are serious limiting factors to *Dacryodes edulis* in Nigeria. Production of this economic crop has been greatly hindered by diseases whose etiology was not known to farmers. However, this study add

information to earlier reports about the role of these fungi in causing diseases of the African pear Ajibesin, (2010).

Materials and Methods

Sampling Area

The study was carried out in Afikpo North Local Government Area, of Ebonyi State, Nigeria. The rainy season begins in April and last till October with annual rainfall of 1500m to 2200mm. An average annual temperature above 22^{o}c (68^{o}F) creates an annual relative humidity of 75% with humidity reaching 90% in the rainy season. The laboratory study was carried out at the microbiology laboratory of Akanu Ibiam Federal Polytechnic, Unwana-Afikpo. A total of 400 *Dacryodes edulis* leaves were sampled. Diseased leaves were used for the sampling. A random sampling method was adopted. In each tree, one hundred (100) diseased leaves situated at mid height of the tree were plucked. The leaves were labeled and placed in aseptic collection bag and were taken to the laboratory for analysis. Leaves were sorted out based on the different diseases observed. The symptoms observed were first described and classified. The percentage incidence of each disease was calculated using the formula;

$$\frac{\text{Total number of diseased leaves}}{\text{Total number of plants observed}} \times \frac{100}{1}$$

The severity of the disease was assessed using a special score chart based on the area of infection.

0% of leaves covered with rot	=	infection
1-20% of leaves covered with rot slight infection	=	1
21-40% of leaves covered with rot moderate infection	=	2
41-60% of leaves covered with rot very extensive infection	=	3
61-80% of leaves covered with rot very extensive infection	=	4
81-100% of leaves covered with rot completely rotted	=	5

Percentage disease severity was calculated by using the formula as proposed by wheeler (1969).

$$\text{Severity} = \frac{\text{sum of numerical rating}}{\text{Total number of observed fruit}} \times \frac{100}{1}$$

Preparation of Culture Media

The media was prepared by dissolving 13g of Sabourauds Dextrose *Agar* in 200ml of distilled water and heated in an electric water bath (GFL Germany) until the agar dissolved. The P^{H} was measured using P^{H}metre (Hana, *et al.*; 1998) to ensure compliance with the standard (5.6 + 0.2). The media was sterilized in an autoclave at 121^{o}c, 15 ibs pressure for 15 minutes. The sterilized media was allowed to cool to about 45^{o}c before it was poured aseptically into sterile petri dishes in 20ml aliquots. The poured plates were allowed to cool and gel before they were used for microbial culture.

Isolation of Microorganisms from Diseased Leaves.

Fungal pathogens responsible for *Dacryodes edulis* leaf's rotting were isolated from the diseased leaves. The diseased tissue was surface sterilized with 70% ethanol for three (3) minutes. A cross incisions in the form of 'V' were made one (1) mm away from the point of disease development with sterile scalpel. A pie of tissue was taken and placed on the prepared media (*Sabour aud Dextrose Agar*) and was incubated at 37^{o}c for 3 days, the isolates were sub-cultured

into pure culture and identified based on their growth habits and spore characteristics with the help of manuals by (Barret *et al.*, 2000, Onyeagba., 2004).

Identification of Fungal Isolates

The isolated organisms were identified by preparing a slide of each organism and viewing it under microscope.

Pathogenicity Test on Leaves

A total of twenty-five (25) healthy *Dacryodes edulis* leaves still attached to the tree in the field were randomly selected for pathogenicity test as proposed by Robbert Koch (1882). They were surface sterilized with hypo *hydrochlorite* rinsed with distilled water and allowed to air dry. Five healthy leaves were used for each isolate at five replications. Razor blade was used to scrap some portions of the leaf surface to expose the protoplast. Same was done to the control. Two drops of spore suspension of isolates were deposited around the wounded spot made on the healthy leaves. In the control experiment, two drops of distilled water were used. The wounded part of inoculated leaf was covered with Vaseline, placed in a sterile transparent polyethene bag and incubated for five (5) days at 25^{o}c. The extent of rot was recorded and calculated for each isolate. Re-isolation was also carried out on the fifth day and noticeable growths were observed.

Results

Rotting Symptoms

Different symptoms were observed on the different tress visited during sampling. The symptoms observed were as follows

Black Leaf Spot

The disease is localized. Leaf spots were characterized by black spots randomly scattered across the leaf. Some spots had the appearance of concentric rings. The diseased leaf defoliates at the early stage. Some leaves shrinked and withered as a result of the disease.

Crinkling of Leaves

Yellowing began to appear on side of the leaf and then leaflet became yellow on the other half of the leaf. As the disease progresses, one side of the leaf shrinks or twins and this process spread to other side as the infection worsens.

White Patches

White patch, also known as white blight, was one of the diseases observed in some leaves of *Dacryodes edulis* in area surveyed. Symptoms appear in white circles or patches up to 1 foot in diameter, and small white or tan mushroom were produced directly on the blighted leaves.

Leaf Yellowing

Leaves with yellowing first turn yellow green to yellow between veins, with the veins remaining darker green become pale yellow and develop brown spots between the main veins.

Disease Incidence and Severity

Disease incidence and severity observed during the field survey were summarized in tables 1 and 2. It was observed that the mean values of the disease obtained from the survey differed significantly when analyzed using analysis of variance (ANOVA).Yellowing had the highest incidence (29.25%) followed by Black leaf spot (24.50%) crinkling 21% with white patches 9.75% having the least disease incidence and was significant at p=0.05

Table 1: Percentage Disease Incidence of *Dacryodes edulis* leaves diseases observed.

	Black Leaf Spot	**Crinkling**	**White Patches**	**Yellowing**
Tree 1	30	16	8	23
Tree 2	25	19	12	42
Tree 3	23	30	7	23
Tree 4	20	19	12	29
Mean	24.50±	21.0±	9.75±	29.25±
	4.20	6.164	2.63	8.95

The disease severity is presented in table 2. Black leaf spot was the most severe of the diseases having 29.22%. This was followed by yellowing 21.09% with white patches 18.43% and crinkling disease having the least severity on the *Dacryodes edulis* leave having 16.09% the result showed there was significant at p=0.5

Table 2: Severity of *Dacryodes Edulis* leaves diseases.

	Black Leaf Spot	**Crinkling**	**White Patches**	**Yellowing**
Tree 1	40	20	25	35
Tree 2	35	15	17.5	22.5
Tree 3	20	7.5	12.5	17.5
Tree 4	21.8	21.8	18.7	9.37
Mean	29.22±	16.0±	18.43±	21.09±
	9.80	6.42	5.14	10.73

Isolated Organisms

The following organisms were constantly isolated from diseased *Dacryodes Edulis* leaves, *Botryodiplodia Theobromae,* Aspergillums Niger, aspergillums *flavus, Rlizopusstoloniferwere* identities according to Barnett, Hunter (1987)

Discussion

Diseases are serious limiting factors to *Dacryodes edulis* (local pear) in Nigeria. Production of this economic crop has beengreatly hindered by diseases whose etiology was not known to farmers. However, this study adds information to earlier reports about the role of these fungi in causing diseases of the African pear Ajibesin, (2010). The ability of the isolates to cause black leaf spots and white patches diseases show that the major disease limiting *Dacryodes edulis* production in Afikpo are black leaf spots and white patches diseases. The pathogens that cause field diseases were isolated and their extent of loss on *Dacryodes edulis* leaves was assessed by systematic survey (table 1). The result of the diseases sampling in the different areas studied showed a high prevalence of diseased leaves of *Dacryodes edulis* leaves inspected. Yellowing had the highest incidence of (29. 250%) then followed by black leaf spot (24. 500%) Crinkling (21.00%) with white patches (9.750%) having the lowest disease incidence. These diseases were found to be serious in some trees as seen in the severity survey. Among the surveyed trees which were in areas at the same *alliude* and with common boundary, two trees were severely affected while the leaves were less affected by the diseases observed in two trees (table 2).

Similar observations were made by Okigbo, (2001) with respect to infection by *Macrophomamangiterae,* the causal agent of mango blight. Many leaf spot diseases manifest more at the outset of the rainy season since the high relative humidity helps in faster development (Derso 1999, Okigho 201) and rainfall aids in the dispersal of fungi spores (Chang*et al.*, 1996). This

confirms the report of Okigbo (2001) that humidity furthers the dispersal of spores of *Macrophomamangiferae.* The susceptibility of individual ire depended on the local cultivars. Shannon (2002) had reported that pathogens infects leaves and fruits during prolonged periods of heavy rainfall and high humidity, especially when plants are overcrowded or over fertilized with nitrogen. The organisms associated with the diseases of *Dacryodes edulis* leaves in the present study were *Botryodiplodia, Theobromal Pat, Aspergillus* Niger, *thiegbem, Aspergillus flavus* Link ex and *Rhizopus stolonifer* went. These organisms have been associated with post-harvest rot (Ogundana*et al.,* 1970, Okigbo, 2002). Fungal pathogens are known to be responsible for the post-harvest deterioration of many tropical fruits *Botryodiplodia theobromae* has been reported to be one of the most important fruit rot pathogens in southwestern Nigeria (Adija and Fajola 1982). Olunloyo (1986) had previously reported the involvement of *Penicilumcitrunum, Aspergillus niger, Fusarium* spp.

Dacryodes edulis leaves pathogen might have probably resided in dead stems and them dispersed by rain splash into the growing leaves to initiate infection spore. It is also possible that insect vectors disperse the pathogens (Sigh 2009). The *pathogenicity* test that only two of the isolates were pathogenic namely; *Aspergillus flavus and Botryodiplidia theobromae* and when re-isolated from the infected materials, the fungi were identified to the initial isolates. *Botryodiplodia theobromae* induced mean rot of 0.96cm within 7 days and *Aspergillus flavus* caused mean rots of 1.10 within the same period.

Onuegbu, (1999) observed a loss of coherence of green and normal cocoyam, corn slices by cell wall-degrading enzymes produced by cocoyam isolates *Botryodiplodia theobromae* pat. *Rhizopus Stolonifer, Penicillum* Specie. *Aspergillus flavus, Sclerotium rolfsii and Fusarium. Chukwuet. al.,* (2002) also observed that *Aspergillus niger, Penicillumitalicum,* reduced the *viscocity* and storage stability of the seed of *Irvingiagabonensis.*

Conclusion

Several disease symptoms were evident in field grown *Dacryodes edulis* in some parts of Afikpo. Foliar symptoms included black leaf spots, yellowing white patches and crinkling diseases. *Botryodiplodiatheobromal* pat. *Aspergillus niger, Aspergillus flavus* link ex and *Rhizopus Stolonifer* were isolated from diseased leaves. Two of the isolates were pathogenic on healthy leaves of *Dacryodes edulis*. The present findings support earlier reports that these organisms are causal agents of disease in the leaves of *Dacryodes edulis.*

Recommendations

The Researcher therefore, recommend that more detailed studies should be carried out on diseases affecting other parts of the plant so as to make a concrete conclusion on the general diseases affecting the plant and the causal agents of the diseases. This will give a more knowledge on how to control disease occurrence.

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